DEVELOPMENT OF THE PULMONARY SURFACTANT SYSTEM IN NON-MAMMALIAN AMNIOTES

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ABSTRACT

Pulmonary surfactant is a complex mixture of phospholipids, neutral lipids and proteins that lines the inner surface of the lung, where it modulates surface tension thereby increasing lung compliance and preventing the transudation of fluid. In mammals, the pulmonary surfactant system develops towards the end of gestation, characterised by an increase in the saturation of phospholipids in lung washings and the appearance of surfactant proteins in amniotic fluid. Birth, the transition from *in utero* to the external environment, is a rapid process. At this time, the pulmonary surfactant system is important in opening and clearing the lungs of fluid to initiate pulmonary ventilation. In oviparous vertebrates, escape from an egg can be a long and exhausting process. The young commence pulmonary ventilation and hatching by "pipping" through the eggshell, where they remain for some time, presumably clearing their lungs. This study describes the development of the pulmonary surfactant system within the non-mammalian amniotes and relates changes in development in response to birth strategy, lung morphology and phylogeny in order to determine the extent of conservation within this developmental process. Total phospholipid (PL), disaturated phospholipid (DSP) and cholesterol (Chol) were quantified from lung washings of embryonic and hatchling chickens, oviparous bearded dragons, viviparous sleepy lizards, snapping turtles and green sea turtles throughout the final stages of incubation and gestation.

In addition to the lipids, another specific parameter was measured for each species to further describe the development of the system. In all cases, the pattern of development of the pulmonary surfactant lipids was consistent with that of mammals. PL and DSP increased throughout the latter stages of development and Chol was differentially regulated from the PLs. Maximal secretion of both PL and DSP occurred at "pipping" in oviparous reptiles, coincident with the onset of airbreathing. Similarly, the amount of DSP relative to total PL was maximal immediately after the initiation of airbreathing in chickens. The

relative timing of the appearance of the lipids differed between groups. In the oviparous lizard, surfactant lipids were released over a relatively shorter time than that of the viviparous lizard, turtles, the chicken and mammals.

The morphology and maturation of the type II cells from bearded dragons matched that of mammals. Surfactant protein (SP)-A messenger RNA was detected in chicken lung tissue throughout development, appearing relatively earlier in development compared to mammals. Unlike the surfactant lipids, the antioxidant enzymes catalase, superoxide dismutase and glutathione peroxidase, did not differ appreciably throughout gestation in the viviparous lizard, suggesting that the pulmonary surfactant system and antioxidant enzyme system develop independently of each other. Expression of SP-B and thyroid transcription factor-1 (TTF-1), a mammalian regulator of cell differentiation and gene expression of surfactant proteins was similar between mammals and the freshwater turtle. Environmental cues, such as hypoxia, did not affect incubation time, absolute, nor relative abundance of the surfactant lipids in sea turtles, demonstrating that the development of the system is robust in this species.

Despite temporal differences and vastly different lung morphologies, birth strategies and phylogenies, the overall development and maturation of the pulmonary surfactant lipids and proteins are highly conserved amongst the amniotes. However, the stimuli for secretion and the development of other systems crucial to airbreathing, such as the antioxidant enzyme system, show greater plasticity throughout evolution.